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Microbiological and immuno-pathological aspects of peri- implant diseases

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Abstract

Peri-implant diseases are a cluster of “contemporary” oral infections in humans that have emerged as a result of the routine application of osseointegrated dental implants in clinical practice. They are characterized by the inflammatory destruction of the implant-supporting tissues, as a result of biofilm formation on the implant surface. Peri-implant mucositis and peri-implantitis are analogous to gingivitis and periodontitis that affect natural teeth. The aim of this comprehensive review was to provide insights into the infectious aetiology and immuno-pathology of peri-implant diseases, and to identify similarities and differences with periodontal diseases. The microbial composition of peri-implantitis-associated biofilms is mixed, non-specific and very similar to that of periodontitis. A considerable exception is the frequent presence of high numbers of staphylococci and enteric bacteria in peri-implantitis. The sequence of immuno-pathological events and the qualitative composition of the immune cells in peri-implant infections are similar to that of periodontal infections. The lesions are characterized predominantly by neutrophils, macrophages, T- and B-cells. Nevertheless, compared to periodontitis, peri-implantitis is marked by a more extensive inflammatory infiltrate and innate immune response, a greater severity of tissue destruction and a faster progression rate. This could well account for the structural differences between the two tissue types, predominantly the lack of periodontal ligament and Sharpey’s fibres around implants. In order to support the early diagnosis and prevention of peri-implantitis, it is crucial to explain its fast progression rate by elucidating the underlying molecular mechanisms. This could be achieved, for instance, by utilizing the non-invasive collection and analysis of peri-implant crevicular fluid.

Oral ecology and biofilm formation

The oral cavity is a dynamic ecosystem continuously colonized by microorganisms which are collectively defined as the oral microbial flora. These have evolved along with the host, and their growth is dependent on the available nutrients and their capacity to withstand local immune defenses. Bacteria grow on natural (tooth, mucosa) or artificial (prostheses, implants) surfaces as biofilms, which are highly organized and structured microbial communities, embedded in polymeric matrices. As part of a biofilm community, bacteria become more virulent than their planktonic forms and less penetrable by elements of the immune system, such as neutrophils and antibodies, or antimicrobial factors (1). The contemporary notion on how oral biofilms are causing oral diseases, such as caries, periodontitis, or peri-implantitis is well summarized by the “ecological plaque hypothesis” (2). According to this hypothesis, it is the interrelationship between the bacteria and the host response that defines health or disease. Changes in the local microenvironment may shift the composition of the biofilm microflora. Under the newly established conditions, the predominant microbial species may display enhanced virulence and act as opportunistic pathogens, causing disease to susceptible hosts. Thus, oral infections are considered to be endogenous infections.

Clinical characteristics of peri-implant diseases

Osseointegrated dental implants are metallic devices made predominantly of titanium that are surgically implanted into the jaw bone, substituting one or more missing teeth. A prosthetic restoration is then fit on a transmucosal abutment structure, aiming to restore the functional and aesthetic needs of that site of the oral cavity. Nevertheless, the

artificial manufactured surfaces of dental implants are also prone to microbial colonization and biofilm formation, eventually causing infection of the implant-supporting (peri-implant) tissues.

Failures of dental implant function can be classified either as early, or as late ones (3, 4). Early implant failures are the ones that occur due to incomplete osseointegration, before or after the functional loading of the implant. Such failures include early loading, surgical contamination, poor compatibility of the implanted material, or inefficient healing. On the other hand, late failures involve disruption of the function of an already osseointegrated implant, mainly due to chronic infection of the peri-implant tissues. In peri-implant mucositis, the biofilm-induced inflammation is localized on the soft peri-implant mucosa, with no evidence of destruction of the supporting bone. In peri-implantitis, the inflammation expands deeper into the bone tissue, leading to its gradual destruction, and eventually to implant loss. These two forms of peri-implant disease are analogous to gingivitis and periodontitis of natural teeth (5).

The diagnostic criteria for peri-implant diseases are mainly clinical and radiographic (6). Peri-implant mucositis is characterized by inflamed or erythematous mucosa and bleeding during the examination. Peri-implantitis is further characterized by the formation of a peri-implant pocket greater than 4 mm, bleeding or suppuration on probing, and, radiographically, a characteristic symmetrical “saucer-shaped” bone destruction (or “crater”) around the implant. Mobility can occur at progressed stages and is associated with poor prognosis of the implant. The increased probing depth, the

positive bleeding on probing and the presence of suppuration in particular are important diagnostic indicators of peri-implant diseases (7).

The consensus risk factors for peri-implantitis are poor oral hygiene, smoking, systemic conditions (e.g. diabetes mellitus), genetic susceptibility, potentially alcohol consumption, and prior history of periodontitis (7). The first four are shared in common with periodontitis, whereas the last one denotes an increased susceptibility to local oral infection. Hence, there appears to be a parallel trend between periodontal and peri-implant diseases.

Aetiology and pathogenesis of peri-implant diseases

There are two crucial steps in understanding the infectious aetiology and pathogenesis of peri-implant diseases: understanding of a) the aetiological factors and pathogenic mechanisms that govern periodontal diseases, and b) the structural and immunopathological differences between periodontal and peri-implant tissues. In other terms, the already established knowledge on periodontal diseases should be a starting point for deciphering in peri-implant diseases, keeping well in view that any identified differences between the two could yield independent research questions.

Differences between periodontal and peri-implant tissues

Although there are in principle clinical and histopathological similarities between the periodontal and peri-implant mucosa, there are also some fundamental differences (5). The main one is the absence of Sharpey's fibres inserting perpendicularly to the implant surface, as opposed to the cementum of natural teeth. Instead, the collagen fibers of the

submucosal connective tissue are arranged parallel to implant surface. This results in the peri-implant crevice being deeper than the gingival crevice, eventually allowing the deeper penetration of bacteria. In terms of the interface with the bone, implants are directly osseointegrated into the bone. On the contrary, natural teeth are socketed into it via the periodontal ligament and the associated Sharpey's fibres at its extremities. The lack of the periodontal ligament poses a number of biological "disadvantages" for the implant, compared to natural teeth. These include a reduced physical barrier against bacterial invasion into the submucosal tissue. Hence the peri-implant tissues present an "open wound" conformation, being more susceptible to an endogenous infection, as compared to periodontal tissues. The lack of periodontal ligament poses yet another disadvantage, which is the restricted blood supply. That is, in the case of the soft peri-implant tissues, blood supply is facilitated via the supra-periosteal vessels and not via the periodontal ligament. This has subsequent effects on reduced presence of nutrients and cells of the immune system, which are needed to tackle the early stages of bacterial establishment and infection. Another potential drawback, which has not yet been extensively considered, is the reduced implant mobility. This may well impair the capacity of the implant to withstand occlusal and masticatory forces.

Peri-implant microbiology

The bacterial colonization of the surface starts already 30 min after implant insertion, and similar bacterial taxa can be identified on the implant after several months (8). The bacterial composition of the biofilm formed on implants closely resembles that of the neighboring teeth (9, 10). Hence, the microbial flora on natural teeth is a "reservoir" for

the biofilms that build-up around implants. In terms of initial (i.e. 4 weeks) subgingival colonization, the frequency of detection of different species is similar between natural teeth and implants. Nevertheless, the colonization pattern on implants appears to be initially slower than on natural teeth (11).

The peri-implant microflora in health consists mainly of Gram-positive cocci and non-motile bacilli, and a limited number Gram-negative anaerobic species, resembling gingival health (12, 13). Nevertheless, the switch to peri-implant mucositis is associated with increased presence of cocci, motile bacilli and spirochetes, at proportions comparable to gingivitis (14). The transition to peri-implantitis is associated mainly with the emergence of Gram-negative, motile, and anaerobic species that are commonly found in periodontitis (12, 15). Reportedly, the microbial flora of the peri-implant pockets resembles that of the neighboring periodontal pockets, while the three “red complex” species, namely *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*, can be found at higher counts in peri-implantitis (9, 16, 17). Collectively, it appears that the qualitative composition of the biofilm microflora in peri-implantitis resembles that of periodontitis, which also implies that patients with active periodontal disease are at higher risk for developing peri-implantitis. Of note, submucosal biofilms obtained from peri-implantitis patients also yield bacteria that display *in vitro* resistance to one or more standard antibiotic treatments. These are most often *Prevotella intermedia/nigrescens* or *Streptococcus constellatus* (18).

Nevertheless, a number of microorganisms have been identified in peri-implantitis that may not be common to periodontitis. These include bacterial species such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterobacter aerogenes*,

Enterobacter cloace, *Escherichia coli*, *Helicobacter pylori*, *Peptostreptococcus micra*, *Pseudomonas* spp, as well as *Candida* spp fungi (15, 19-25). Presence of *S. aureus* shortly after implant insertion can be confirmed even one year later (10). It has also been shown that up-to 18.6% of peri-implantitis lesions harbor aerobic Gram-negative bacilli, such as enteric rods and coliforms, or non-enteric rods, but the microbial burden may not fully correspond to disease severity (26). In a dog model of ligature-induced peri-implantitis, analysis of the microbial profiles after 3 months revealed an increase in total bacterial loads, predominated by an anaerobic Gram-negative microflora. The large variation of the microbial profiles hindered the interpretation of any association with disease progression (27).

An important question raised with regards to peri-implant microbiota, is whether there are differences between fully and partially edentulous patients bearing implants. Bacterial colonizers of healthy implants in fully edentulous individuals, appear to be similar to those found at healthy periodontal sites. In partially edentulous patients, the implant surface is colonized by the same species as the neighboring teeth, soft tissues, tongue and saliva (28, 29). Nevertheless, putative periodontal pathogens may be detected at higher proportions (30), prevalence and numbers (31), than in fully edentulous patients. Early studies indicated absence of detection of *Aggregatibacter actinomycetemcomitans* and *P. gingivalis*, which could allude to the subgingival niche being their source (32, 33). However, later studies indicated that these taxa can be detected in peri-implantitis occurring in fully edentulous patients (11, 16, 34), indicating that they are not harbored solely in the subgingival region. This means that they are also found at other niches of the oral cavity, such as the soft tissues or saliva, and they

are capable of colonizing the pristine implants or the peri-implant tissues of fully edentulous patients. Hence, bacterial species harbored in subgingival biofilms cannot be completely eliminated after tooth extraction, but may re-surface to colonize the implants.

Considerable debate is made regarding the implant surface characteristics and its potential for biofilm formation. It is not clear if the microbial composition of a biofilm is affected by the physicochemical properties and texture characteristics of the implant surface. Higher roughness and higher free energy of the implant surface may favour biofilm formation (35), whereas peri-implantitis may occur earlier, with a faster and more extensive progression in implants with rougher surface (36-38). Nevertheless, it is also shown that abutments with different surface characteristics can influence neither biofilm formation on the implant surface, nor the extent and cellular composition of the resulting inflammatory lesion (39). Moreover, no implant system or surface type was found to be superior in terms of marginal bone preservation (40). However, one should consider that the implant surface, like the surface of natural teeth, is immediately covered by salivary mucoproteins, which are imperative for bacterial adhesion (41). These are genetically defined in each individual, and hence the same proteins that coat natural teeth and implants can be recognized by the same bacterial species. In this respect, potential differences in bacterial adhesion due to surface microstructure may partially be equilibrated by the mediating salivary pellicle (42). Hence, given the biological involvement of the pellicle, implant surface characteristics may not notably affect the initial stages of biofilm formation and composition.

Pathogenesis of peri-implant diseases

Like in natural teeth, the accumulation of biofilm on the implant surface favors the initiation of tissue inflammation. At an initial stage, peri-implant mucositis is established, whereas spread of inflammation towards the supportive bone leads to peri-implantitis. The information available to-date comes mainly from comparative studies using human biopsy material from peri-implant mucosa, as well as experimental studies in Beagle dogs. Peri-implant mucositis is characterized by inflammation that culminates to an acanthotic epithelium, connective tissue loss, microvascular changes (43), and increased infiltration of T- and B-cells, neutrophils and macrophages (44, 45). These events are similar to gingivitis, but of greater magnitude (46-49). Of note, compared to the gingival mucosa, the peri-implant mucosa may present less Langerhan's cells and more interstitial dendritic cells (50). The processing of the implant surface may also play a role in the inflammatory response of the adjacent peri-implant mucosal tissue. For instance, peri-implant mucosa biopsies obtained around acid-etched titanium healing caps exhibit greater microvessel density and inflammatory infiltrate, including higher number of T- and B-cells, compared to machined caps (51). These histopathological characteristics are commensurate with a more pronounced inflammatory response.

The swift to peri-implantitis is characterized by even higher proportions of neutrophils, macrophages, T- and B-cells, than peri-implant mucositis or periodontitis (52, 53). A global transcriptome (microarray) analysis of human biopsies showed both shared and distinct gene expression "signatures" between peri-implantitis and periodontitis (54). Comparative experimental studies in Beagle dogs demonstrate loss of connective tissue and establishment of an inflammatory infiltrate in both periodontitis

and peri-implantitis (44, 48). However, the extent of the inflammatory infiltrate is greater in peri-implantitis, spreading towards the bone marrow (47). A larger proportion of neutrophils and osteoclasts is also observed in peri-implantitis, compared to periodontitis (38).

The innate immune responses of the soft connective tissue may also differ between periodontitis and peri-implantitis. Granulation tissue from peri-implantitis sites exhibits higher mRNA expression of pro-inflammatory cytokines Interleukin(IL)-6, IL-8 and tumor necrosis factor (TNF)- α , compared to matched tissue from periodontitis sites (55). Immunohistochemical comparison revealed that IL-1 α staining was more prevalent in peri-implantitis tissue, while TNF- α was more prevalent in periodontitis tissue (56). Interestingly, fibroblasts isolated from peri-implantitis granulation tissue exhibited enhanced production of vascularization factors, matrix metalloproteases and complement receptor C1q, as well as reduced production of inhibitors of metalloproteases and growth factors that stimulate collagen synthesis, compared to fibroblasts from periodontitis granulation tissue. This specialized innate immune response of the peri-implant connective tissue may promote the migration and maintenance of inflammatory cell infiltrates into the affected site (57, 58).

Hence, it appears that the sequence of inflammatory events and qualitative composition of the immune cells in peri-implantitis is rather similar to periodontitis (59). What differentiates peri-implantitis is the higher proportion of immune cells and associated inflammatory mediators, and the larger infiltrate that expands apically to the junctional epithelium towards the bone marrow (5, 60).

Although histopathologically peri-implant infections are quite well described, the molecular events that govern these processes are not yet fully characterized. Varying reports have attempted to associate specific genotypes of the immune response with peri-implantitis. There is some evidence that IL-1 polymorphisms, particularly in conjunction to smoking, may confer an increased risk for peri-implant bone loss or implant loss (61, 62), but this may not adequately reflect the produced levels of inflammatory mediators (63-65). Therefore, to-date no specific genotype or systemic inflammatory marker exists that can reliably indicate peri-implant disease progression or susceptibility (66).

Nevertheless, on the level of the affected site, there is good merit to investigate the molecular content of peri-implant crevicular fluid (PICF), which is the inflammatory exudate of the peri-implant sulcus, in an analogous fashion as the gingival crevicular fluid (GCF) of natural teeth. Candidate molecules can be the ones already validated in periodontitis, such as the matrix metalloproteinases (67, 68), or the receptor activator of NF-kappaB ligand (RANKL) - osteoprotegerin (OPG) system (69, 70), which show a positive association with disease occurrence and severity (71, 72). An increasing stream of evidence shows the association of these factors with peri-implantitis (73-83). Moreover, higher concentrations of TNF- α , IL-17, IL-1 β and nitric oxide are demonstrated in PICF of patients with peri-implantitis, compared to healthy controls (84-87), whereas no differences were detected with regard to IL-6, IL-8, IL-10 (85), or prostaglandin E₂ (88). These findings require further investigation, as they could potentially be used in the development of molecular diagnostic utilities and targets for the immuno-modulatory treatment of peri-implant diseases.

Synopsis of peri-implant diseases

In summary, biofilms can form on implant surfaces, similarly to natural teeth, causing inflammation and subsequent destruction of the surrounding tissues. The microbial “reservoir” for the contamination of the implant surface includes neighboring teeth, periodontal pockets, saliva and soft oral tissues. Peri-implantitis appears to be a non-specific mixed-flora infection, exhibiting similar microbiological characteristics to periodontitis, with the exception of staphylococci, peptostreptococci, enterobacteria and *Candida* spp. The sequence of immune-pathological events and qualitative composition of immune cells and inflammatory response in peri-implant infections is similar to that of periodontal infections. Nevertheless, the inflammatory tissue destruction in peri-implantitis is faster and more extensive than in periodontitis. This could well account for structural differences in the conformation of the two tissue types. Hence peri-implant diseases are endogenous oral infections that have co-emerged with the routine application of osseointegrated dental implants, as part of restorative dental treatment. There is a clear need for a better understanding of these “contemporary” oral diseases, and a careful consideration prior to treatment planning, both by clinicians and patients.

Conflicts of Interest statement

The Author declares no conflicts of interest. This study was supported by the Author’s Institute.

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